THE COLORIMETRIC ESTIMATION OF MORPHINE IN CAMPHORATED TINCTURE OF OPIUM

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The official assay for morphine in camphorated tincture of opium utilises the measurement of the yellowish-brown colour produced by the reaction of morphine with a nitrite in acid solution, followed by the addition of excess of alkali. This colour reaction was first reported by Radulescu[1], and later used as a quantitative method by Fabinyi[2] while van Arkel[3] made the production of the colour more reliable by controlling the acidity and time of reaction, but claimed no great accuracy. Allport and Jones[4] evolved a rapid estimation using a tintometer and claimed an accuracy of ± 10 per cent.

Adamson and Handisyde[5] investigating the method by use of a Spekker absorptiometer reported that the length of time for the reaction in acid solution had a marked effect upon the depth of colour produced. The same authors[6] later critically reviewed the conditions for the production of the colour as applied to the official method, and recommended a time for reaction of exactly 15 minutes, in a specified volume of 0·1 N hydrochloric acid. Under these conditions they gave the method an accuracy of ± 2 per cent. Stephens[7] has given further information on the reaction, particularly with regard to the effect of temperature.

As to the specificity of the reaction when applied to the estimation of morphine, Radulescu[8] examined some 150 vegetable extracts, but found only one of doubtful origin which gave the same reaction. Adamson and Handisyde[5] reported that narcotine, papaverine and codeine do not give a reaction, but the same authors[6] stated that it is given generally by other phenols and substances containing a phenolic group. It is interesting to note in this connection that Nicholls[9] found that Mannich's method for the estimation of morphine, which utilises the precipitation of morphine as its dinitrophenyl ester, was not specific when applied to opium and its products owing to excessive interference from other phenolic alkaloids present.

The official method now in use consists essentially of the evaporation of a portion of the tincture, extraction of the residue with lime water, followed by extraction of the lime water extract with ether. After reduction of the *p*H of the aqueous solution with ammonium sulphate the morphine is extracted with ethanol and chloroform, the mixed solvents are removed, and the residue dissolved in acid. The acid solution is diluted to a definite volume to render it 0·1 N and a portion is used for the development of the colour with nitrite and ammonia, which is then measured by comparison with a standard morphine-nitrite reagent.

It has been noticed in this laboratory, that the official assay of

camphorated tincture of opium gives results significantly higher than expected. Allowing no error in manufacture and assuming the official assay for tincture of opium to be correct, camphorated tincture of opium which contains 1/20th of its volume of tincture of opium should contain between 0·0475 to 0·0525 per cent. of morphine. The official limits are 0·045 to 0·055 per cent. Some results showing discrepancies ranging between + 6 and + 20 per cent. are given in Table I. The quantity of morphine calculated to be present in each batch of camphorated tincture of opium is derived from the strength of the particular batch

TABLE I

Tincture of Opium		Camphorated tincture of Opium		
Batch	Morphine by Assay	Batch	Morphine calculated	Morphine by Assay
	per cent.		per cent.	per cent.
61	0·97	260	0·049	0·055
62	0·95	266	0·048	0·058
63	1·05	273	0·053	0·056
64	0·98	278	0·049	0·058
65	0·97	280	0·049	0·059

of tincture of opium used in its preparation shown on the same line in the table. It had been noted frequently too that if the final solution in 0·1 N hydrochloric acid were boiled, a red colour was produced. It was decided to examine the official method to see if an interfering substance, possibly associated with the red colour, could be isolated and subsequently removed.

In the following experiments the optical density of the colours produced was measured on a Spekker absorptiometer in a 4 cm. cell using OBI glass filters, and the equivalent quantity of morphine estimated by reference to a standard curve prepared under the same conditions. In all cases a "blank" containing the same quantity of the extracted morphine in 0·1 N acid solution, with the ammonia added before the nitrite, was used for comparison.

Experimental

Effect of Camphor, benzoic acid and oil of anise. Samples were prepared—(1) As the official preparation, (2) As the official preparation omitting camphor, benzoic acid and anise oil, (3) As the official preparation substituting an equivalent quantity of morphine for the tincture of opium, and (4) As the official preparation omitting the tincture of opium. They were made to contain (where applicable) a calculated content of 0·050 per cent. of morphine.

The official assay was performed on each sample, and it was found that there was no interference due to camphor, benzioc acid or anise oil. In the samples containing tincture of opium as the source of morphine, results 10 to 12 per cent. higher than theory were obtained, whilst in sample (3), the recovery of pure morphine was complete and reproducible to ± 2 per cent. Where no source of morphine was included in sample 4, there was no recovery of "apparent" morphine. These results are given in Table II.

The ether extractions were examined to see if they contained a substance which gave Radulescu's reaction and if this were the case, to attempt further ether extractions under these conditions, to effect its complete removal. The ether layers were evaporated and dissolved in 0·1 N hydrochloric acid, but it was found that these solutions gave no colour with nitrite and ammonia. However it was found in the case of sample 1 and 2, that if the acid solution was first boiled, no red colour was produced, but the solution then gave a reaction with nitrite and ammonia. The colour was equivalent to about 8 per cent. of the morphine content of the samples. Further ether extraction yielded no colour after evaporation and boiling with 0·1 N acid. It would appear that there is a substance originating in the tincture of opium which gives Radulescu's reaction after boiling with 0·1 N acid, but no red colour, and is completely removed by the two extractions with ether.

TABLE II

(1) Official preparation	(2) Official preparation omitting camphor, etc.	(3) Preparation using pure morphine	(4) Official preparation omitting tincture of opium
per cent.	per cent.	per cent.	per cent.
0·056	0·056	0·050	0·000
0·056	0·055	0·049	0·000
0·055	0·056	0·050	0·000
0·056	0·056	0·050	0·000

Extraction with ether at reduced p*H.* Garratt[10] in an examination of the colorimetric estimation of morphine in aromatic powder of chalk with opium, stated that phenolic and non-phenolic substances, present in the aromatic portion of the preparation gave a colour but that they may be removed by extraction with 3 quantities of ether after the addition of ammonium sulphate. This method of extraction was tried, in addition to the preliminary ether extractions, which, as mentioned above, extracted one type of interfering substance. It was found that a further substance which gave Radulescu's reaction and also a red colour on boiling with dilute hydrochloric acid was extracted. The conditions were varied but it was found that if the interfering substance (as indicated by the red colour given with hot dilute hydrochloric acid) were to be extracted completely, some of the true morphine content was also extracted. If the conditions were arranged so that an insignificant quantity of morphine was extracted, some of the interfering substance was left behind. The two sets of conditions were—(1) For complete extraction of the interfering substance as indicated by the red colour with hot dilute hydrochloric acid. Extract with 3 quantities of ether, each of 10 ml., mix the extracts and wash with 5 ml. of water, discard the ether and extract the mixed aqueous layers with chloroform and ethanol mixture. (2) For the removal of an insignificant quantity of morphine. Extract with 3 quantities, each of 5 ml., mix the extracts of ether, and wash with 2 separate 5 ml. quantities of water. Discard the ether layer and extract the mixed aqueous layer with chloroform and ethanol mixture. Table

III gives the results obtained by the two methods on samples containing an equivalent morphine content of 0·050 per cent.

TABLE III

First method of extraction				Second method of extraction			
Official preparation		Preparation containing pure morphine		Official preparation		Preparation containing pure morphine	
Morphine by assay	Equivalent morphine in ether layer	Morphine by assay	Equivalent morphine in ether layer	Morphine by assay	Equivalent morphine in ether layer	Morphine by assay	Equivalent morphine in ether layer
per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
0·048	0·0086	0·047	0·0025	0·052	0·0041	0·049	0·0009
0·047	0·0089	0·047	0·0025	0·052	0·0043	0·049	0·0008
0·048	0·0084	0·046	0·0027	0·053	0·0040	0·048	0·0012
0·048	0·0086	0·047	0·0026	0·053	0·0039	0·049	0·0009
0·048	0·0086	0·047	0·0027	0·052	0·0041	0·049	0·0010

Extraction with Benzene. As the method of extraction at reduced *p*H was effective in removing the interfering substance and inaccurate only because of the solubility of morphine in ether, a search was made for another organic solvent in which morphine was less soluble. Tickle[11] gives the partition of morphine in aqueous solution with a number of organic solvents. Of those mentioned, benzene was considered the best to use as the solubility of morphine in this solvent is minimal. It was found that benzene was effective in extracting the substance which gave Radulescu's reaction and also a red colour on boiling with 0·1 N hydrochloric acid. Two extractions with 10 ml. quantities of benzene were found to be sufficient, but troublesome emulsification took place. It was found that the use of 2 quantities, each of 25 ml. of benzene eliminated the emulsification and removed less than 1 per cent. of the morphine. Subsequently a single extraction with 25 ml. of benzene was found to remove the interfering substance effectively. The results obtained using one 25 ml. benzene extraction, which was washed with 5 ml. of water, are given in Table IV. The samples were made to contain 0·050 per cent. of morphine.

TABLE IV

Official preparation		Preparation containing pure morphine	
Morphine by assay	Equivalent morphine in benzene layer	Morphine by assay	Equivalent morphine in benzene layer
per cent.	per cent.	per cent.	per cent.
0·049	0·0064	0·050	0·0003
0·050	0·0062	0·049	0·0003
0·050	0·0062	0·050	0·0002
0·050	0·0062	0·050	0·0002
0·049	0·0064	0·050	0·0002

As a simplification, direct estimation of the morphine after the ether and benzene washings without the chloroform-ethanolic extraction was attempted, but it was found that the results obtained were some 10 to 15 per cent. higher than was theoretically predicted. This was confirmed by an examination of the aqueous layer after extraction with chloroform-ethanol which yielded no morphine on further extraction but which itself

when acidified, gave a brown colour with nitrite and ammonia deeper than the very slight colour already present.

Recommended modification. The interfering substance may be removed by inserting in the official method for the assay of camphorated tincture of opium: after the addition of 0·15 g. of ammonium sulphate ". . . extract the aqueous layer with 25 ml. of benzene, separate, wash the benzene with 5 ml. of water and combine the aqueous layers. Discard the benzene and extract the aqueous layer (which now measures 30 ml.) with a mixture of 30 ml. of chloroform and 30 ml. of alcohol. . . ." Results obtained by this modified method for a number of production batches are given in Table V. Samples marked with an asterisk are from other sources of manufacture.

TABLE V

Sample	Official assay	Modified assay	Calculated
	per cent.	per cent.	per cent.
1	0·058	0·050 0·050 0·049	0·050
2	0·063	0·050 0·050 0·050	0·049
3	0·056	0·049 0·048 0·049	0·0485
4*	0·057	0·053	—
5*	0·053	0·048	—
6*	0·055	0·049	—
7*	0·065	0·055	—

Examination of interfering substance.—(1) The morphine-nitrite colour is yellowish-brown, whereas the nitrite colour of the interfering substance may best be described as being yellow. (2) On boiling a hydrochloric acid solution of the interfering substance a red colour is produced and the depth of colour produced with nitrite and ammonia is increased. (3) Morphine may be completely removed from solution in ethanol by a column of ionic exchange resin amberlite IRA400 (OH) but it was found that an ethanolic solution of the interfering substance was not affected.

Even though it has not been proved as being the interfering substance, an alkaloid present in opium which gives a red colour on boiling with dilute acid is rhœadine. The substance extracted by the preliminary ether extractions may be thebaine which is converted into thebanine (having phenolic groups) by the action of dilute hydrochloric acid.

SUMMARY

(1) A substance, other than morphine, which gives Radulescu's reaction and interferes with the colorimetric method of assay for camphorated tincture of opium was detected.

(2) A method which effectively removes the substance without significant loss of morphine, and with the minimum of alteration to the official method is given.

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DISCUSSION

The paper was presented by MR. G. TUNSTALL.

MR. R. L. STEPHENS (Brighton) asked whether the authors had applied their method to the determination of morphine in Tinct. Chlorof. et Morph.

DR. G. E. FOSTER (Dartford) said that the variations reported by the authors and others in the morphine content of camphorated tincture of opium compared with that of the tincture of opium from which it is made were due to the assays for the two preparations being conducted by different methods. If the authors had succeeded in solving the problem by extraction with benzene, they would earn the gratitude of a great number of analysts.

MR. G. TUNSTALL, in reply, said that he had not applied the method to the determination of morphine in Tinct. Chlorof. et Morph. The method had been used to determine morphine in tincture of opium and results were within $\pm$ 1 per cent. of those obtained using the official assay.